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High Prevalence of Thrombocytopenia in SLE Patients With a High Level of Anticardiolipin Antibodies Combined With Lupus Anticoagulant

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The relationship between thrombocytopenia and the level of anticardiolipin antibodies (aCL) and/or the existence of lupus anticoagulant (LA) ware studied in 146 patients with systemic lupus erythematosus (SLE). These patients were divided into six groups: A, those LA positive with a high level of aCL (>10 U/ml) (10 cases); B, those LA positive with a low level of aCL (3–10 U/ml) (15 cases); C, those LA positive but aCL negative (<3 U/ml) (12 cases); D, LA negatives with a high level of aCL (12 cases); E, LA negatives with a low level of aCL (16 cases); and F, aCL and LA double negatives (81 cases). The prevalence of thrombocytopenia (platelet count $\leqq 100 \times 10^9$/L) was by far the highest in group A (9/10 cases, 90.0%, $P < 0.005$, Fisher's exact probability test) as compared with group B (4/15 cases, 26.7%), group C (4/12 cases, 33.3%), group D (1/12 cases, 8.3%), group E (4/16 cases, 25.5%), and group F (9/81 cases, 11.1%).

When the relationship between moderate thrombocytopenia and arterial or venous thrombosis was studied in these patients with SLE, thrombocytopenia was detected in 10 (83.3%, $P < 0.005$, Fisher's exact probability test) of 12 patients with arterial thrombosis; however, it was present in only 4 (23.5%) of 17 patients with venous thrombosis and in 14 (12.3%) of 114 patients without thrombosis. These findings suggest that a high aCL activity combined with LA positively reflects a high risk for both thrombocytopenia and arterial thrombosis. Am. J. Hematol 58:55–60, 1998.

Key words: thrombocytopenia; thrombosis; anticardiolipin antibodies; lupus anticoagulant; systemic lupus erythematosus

INTRODUCTION

Thrombocytopenia and thrombosis are well-known complications of systemic lupus erythematosus (SLE) [1–3]. These complications have been reported to be associated with anticardiolipin antibodies (aCL) and/or lupus anticoagulant (LA) in patients with SLE [4–11]. However, the association between the pathway of platelet activation and/or destruction with aCL and/or LA is not yet well known. The prevalence of thrombosis is reported to not be associated with mild thrombocytopenia [12].

When we divided patients with SLE into four groups: aCL single positive, LA single positive, aCL and LA double positive, and double negative patients, we found that the prevalence of thrombosis was higher in the aCL and LA double positive patients than in the other groups. Furthermore, in these double positive patients, all patients with a high positive level of aCL (above 10 U/ml) had an arterial thrombosis, whereas most of the patients with a lower positive level of aCL (3–10 U/ml) had a venous thrombosis [13].

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In this report, we studied not only the relationship between the incidence of thrombocytopenia and the level of aCL and/or existence of LA, but also the frequency of thrombocytopenia in SLE patients with arterial or venous thrombosis.

MATERIALS AND METHODS

Patients

We studied plasma samples from 146 patients (132 female, 14 male, aged 14–74 years, mean 39.8 years) with systemic lupus erythematosus (SLE). As a control group, we studied 150 normal volunteers (62 female, 88 male) for levels of aCL. Diagnosis of SLE was made according to the revised criteria of the American Rheumatism Association. Thrombocytopenia was defined by a platelet count below 100×10^9/L measured on two separate occasions.

Plasma Samples

Blood was collected into vacuated tubes (5.0 ml total volume tube) containing 0.5 ml of 3.13% trisodium citrate. Plasma was separated immediately by centrifugation at 2,800*g* for 10 min.

Detection of Anticardiolipin Antibodies (aCL)

Recently, it was reported that β2-glycoprotein I (β2-GP I) is an important epitope recognized by aCL and that it depresses cardiolipin binding by antibodies associated with syphilis [10,14–18]. Therefore we measured the levels of aCL with and without β2-GP I as reported previously [13]. Briefly, the aCL levels were measured by a standard enzyme linked immuno-sorbent assay [19]. The cardiolipin-coated wells were washed three times with 250 μl of PBS, pH 7.4, containing 0.05% Tween 20 (PBS-Tween). The wells were then incubated with 50 μl of purified human β2-glycoprotein I (30 μg/ml) (β2-GP I^+ wells) or with 50 μl of 0.5% BSA-PBS (β2-GP I^- wells) for 30 min at room temperature. After incubation, 50 μl of plasma sample (diluted 101 times with 0.5% BSA-PBS) was added to both β2-GP I^+ and β2-GP I^- wells. To exclude the effect of β2-GP I present in patients' plasma, we diluted plasma samples 101 times with PBS. In this way measurement of aCL was not influenced by the β2-GP I in the patients' plasma, because the final concentration of β2-GP I there was below 1 μg/ml. A 50-μl volume of cardiolipin standard sera was added to β2-GP I^+ wells. Following 60 min of incubation at room temperature, the wells were washed three times with PBS-Tween and then incubated with 100 μl of peroxidase-labeled anti-human IgG for 30 min at room temperature. The wells were then washed three times with PBS-Tween, and 100 μl of 0.3 mM tetramethylbenzidine solution containing 0.003% of H_2O_2 was added to each well. After 30 min of incubation, the reaction was terminated by the addition of 100 μl of 2N H_2SO_4, and the OD was measured at 450 nm. The levels of aCL were derived from the calibration curve obtained with β2-GP I^+ wells. The OD levels of each sample in β2-GP I^+ and β2-GP I^- wells were compared for the evaluation of β2-GP I dependency of aCL binding. In this experiment, all of the samples in β2-GP I^+ wells showed higher levels of OD compared with those in β2-GP I^- wells, suggesting that none of the aCL in our samples was associated with syphilis; and so we evaluated the levels of aCL using β2-GP I^+ wells.

Detection of Lupus Anticoagulant (LA)

The presence of LA activity was detected by use of both the Tissue Thromboplastin Inhibition test (TTIT) [20] and a commercial kit [21] (STACLOT LA test, Diagnostica Stago, Asnières-sur-Seine, France). It has been reported that the sensitivity of the TTIT is more accurate than that of the STACLOT LA test, but the specificity of the STACLOT LA test is higher than that of the TTIT [20,21].

Tissue Thromboplastin Inhibition Test (TTIT)

A modified version of the TTIT was used as follows: Initially 100 μl of plasma sample was incubated at 37°C. Following 5 min of incubation, 200 μl of Thromboplastin C (Dade Diagnostics of P.R. Inc.,), which had been preincubated at 37°C, was added, and the clotting time (CT) was then measured with an Amelung KC-10A (Heinrich Amelung GmbH, Lemgo, Germany). This time was designated as CT1.

Thromboplastin C was diluted at 1:50 with 12 mM $CaCl_2$ and incubated at 37°C for 5 min (TTIT-reagent). A 100-μl volume of the plasma sample was incubated at 37°C. Following 5 min of incubation, 200 μl of TTIT-reagent was added, and the clotting time was then measured with the Amelung KC-10A. This time was designated CT2. The ratio of the clotting time (CT2/CT1) was calculated, and a ratio greater than 3.0 was defined as TTIT positive.

STACLOT LA Test

Initially 50 μl of plasma sample was incubated at 37°C with 50 μl of buffer in Tube 1. In Tube 2, a similar volume of plasma sample was incubated with 50 μl of hexagonal phase phosphatidylethanolamine (HPE). Following 9 min of incubation at 37°C, 50 μl of normal human platelet poor plasma was added to each tube. After 1 min of incubation, 100 μl of APTT reagent was added to Tube 1 and Tube 2, and these were incubated for 5 min at 37°C. Then, 100 μl of 0.025M $CaCl_2$, which had been preincubated at 37°C, was added to Tube 1 and Tube 2. The clotting time for Tube 1 and Tube 2 was measured, and when the clotting time in Tube 2 was

greater (8 sec or more) than that in Tube 1, LA was defined as positive.

Determination of Platelet-Associated IgG

Platelet-associated IgG (PAIgG) was quantified by the use of a solid-phase enzyme immuno-assay, as described before [22]. Briefly, blood samples from 31 patients with thrombocytopenia were collected into plastic tubes containing EDTA-2Na. The tubes were centrifuged for 15 min at 450*g* to obtain platelet-rich plasma. The platelet-rich plasma suspended in a solution of 0.3% bovine serum albumin in 9 mM EDTA-PBS (0.01M phosphate buffer 0.15M saline), pH 6.2. The platelets were washed four times for 10 min each time at 1,400*g* with EDTA-PBS buffer and resuspended at 2.5×10^7/ml in 0.1% BSA-PBS, pH 7.2. Platelet counts were determined with a Coulter (Hialeah, FL) Counter. For coating of polystyrene tubes with IgG, 0.5 ml of an IgG solution in (2.5 μg/ml [including 7.5 μg/ml of BSA] of human IgG dissolved in 0.2M carbonate bicarbonate buffer, pH 9.2) was poured in each tube, which was then stored at 4°C overnight. Control tubes used to measure nonspecific binding were incubated with 0.5 ml of BSA (10 μg/ml) in carbonate bicarbonate buffer. After discarding of the solution, 0.5 ml of 1% BSA-PBS (pH 7.2) was added to each tube, which was then incubated for 10 min and washed three times with 0.1% BSA-PBS (pH 7.2). A 0.2 ml volume of platelet suspension or known amounts of human IgG was added to each tube. Simultaneously, 0.1 ml of peroxidase-conjugated antiserum, diluted 1:2,500 with 0.1% BSA-PBS, was added to each tube, which was subsequently incubated for 20 hr at 4°C. After aspiration of the contents, the tubes were washed three times with 0.1% BSA-PBS. One hundred milligrams of 5-aminosalicylic acid and 100 μl of 3% H_2O_2 were diluted just before use in 100 ml of 0.02M phosphate buffer, pH 6.0. A 2.4-ml volume of this solution was added to the tubes, which were incubated for 1 hr at 37°C with stirring every 15 min. The reaction was stopped by the addition of 2 ml of NaN3. Absorbance of each tube was measured at 450 nm. The amount of PAIgG was determined from the absorbance of sample tubes subtracted from that of the control tubes, by use of a standard curve.

Statistical Analysis

The Chi-square test, Fisher's exact probability test, and Pearson's correlation coefficient were used whenever appropriate.

RESULTS

aCL Levels in Normal Healthy Controls

The mean level of aCL was 0.826 ± 0.362 U/ml in 150 normal plasma samples. The valve of 3.0 U/ml of aCL, which was the mean + 6SD of the normal control valve, was decided as the normal cut-off point in this study.

Incidence of LA in Patients With SLE

Using the method of TTIT, LA was detected in 40 of 146 patients with SLE. Thirty-seven of them were confirmed as LA positive by the STACLOT LA test, but 3 of them were negative. Since it was revealed that these 3 patients had a deficiency of coagulation factors, these 3 patients were judged as LA negative.

Prevalence of Thrombocytopenia in Patients With SLE

Thirty-one (21.2%) of the 146 patients with SLE had thrombocytopenia: 28 of these 31 patients had moderate thrombocytopenia (platelet count 50–100 × 10^9/L), and three severe thrombocytopenia (platelet count <50 × 10^9/L) (Table I). Platelet-associated IgG was determined in the 31 patients with thrombocytopenia, and was detected in all 3 patients with severe thrombocytopenia, but not in any of the 28 patients with moderate thrombocytopenia.

Relation Between Thrombocytopenia and aCL and/or LA

Previously, we found that the prevalence of arterial and/or venous thrombosis in the patients with a high positive level of aCL (above 10 U/ml) was quite different from that in the patients with a lower positive level of aCL (3–10 U/ml) in the aCL and LA double positive patients group [13]. Therefore, the 146 patients with SLE were divided into six groups: A, LA positive with a high level of aCL (>10 U/ml) (10 cases); B, LA positive with a low level of aCL (3–10 U/ml) (15 cases); C, LA positive with aCL negative (<3 U/ml) (12 cases); D, LA negative with a high level of aCL (12 cases); E, LA negative with a low level of aCL in LA (16 cases); and F, aCL and LA double negative (81 cases).

The prevalence of thrombocytopenia was 90.0% (9/10 cases, $P < 0.005$, Fisher's exact probability test) in group A, 26.7% (4/15 cases) in group B, 33.3% (4/12 cases) in group C, 8.3% (1/12 cases) in group D, 25.5% (4/16 cases) in group E, and 11.1% (9/81 cases) in group F (Fig. 1). There was a clear negative correlation between platelet count and aCL levels in the LA-positive patient group ($r = -0.489$, $P < 0.005$, Pearson's correlation coefficient, Fig. 2). No correlation was found between platelet count and aCL levels in the LA-negative patient group ($r = -0.035$).

Relation Between Thrombocytopenia and Arterial or Venous Thrombosis

Twelve of 146 patients with SLE had arterial thrombosis (8 cerebral infarction, 1 transient cerebral ischemic attack, 2 femoral artery thrombosis, 1 finger and foot artery thrombosis), and 17 of them had venous thrombo-

TABLE I. Clinical Characteristics of 31 SLE Patients With Thrombocytopenia*

Groups	Patients	Age/sex	aCL (U/ml)	LA	Anti-platelet antibody	Platelet count ($\times 10^9$/L)	Thromboemblic complications
A	1	27/F	176.1	+	−	61	Cerebral infarction
	2	38/M	66.9	+	−	88	Cerebral infarction
	3	47/F	82.2	+	−	59	Cerebral infarction
	4	42/M	115.2	+	−	67	Cerebral infarction
	5	46/F	113.0	+	−	87	Cerebral infarction
	6	41/F	27.1	+	−	98	Cerebral infarction
	7	29/F	366.1	+	−	78	Cerebral infarction
	8	27/M	125.0	+	−	77	TIA
	9	35/F	95.8	+	−	77	Femoral artery thrombosis
B	10	42/F	5.2	+	−	99	Femoral artery thrombosis
	11	36/F	3.8	+	−	95	Renal vein thrombosis
	12	47/F	7.3	+	−	53	Thrombophlebitis
	13	36/M	8.5	+	−	63	Thrombophlebitis
C	14	18/M	1.1	+	−	64	None
	15	60/F	1.0	+	−	59	None
	16	69/F	0.3	+	−	71	None
	17	65/F	0.6	+	+	37	None
D	18	33/F	38.5	−	−	97	None
E	19	45/F	3.4	−	−	84	None
	20	68/F	3.2	−	−	71	None
	21	44/F	6.0	−	−	55	None
	22	30/F	3.0	−	ND	71	Thrombophlebitis
F	23	45/F	0.5	−	−	52	None
	24	49/F	1.9	−	−	57	None
	25	74/F	0.7	−	−	65	None
	26	33/F	0.6	−	−	78	None
	27	32/F	2.0	−	−	96	None
	28	51/F	0.7	−	+	27	None
	29	51/F	0.6	−	+	27	None
	30	58/F	0.8	−	ND	83	None
	31	49/M	0.4	−	ND	70	None

*Group A: aCL (>10 U/ml) LA+; B: aCL (3–10 U/ml) LA+; C: aCL (<3 U/ml) LA+; D: aCL (>10 U/ml) LA−; E: aCL (3–10 U/ml) LA−; F: aCL (<3 U/ml) LA−. TIA: transient cerebral ischemic attack; ND: not determined.

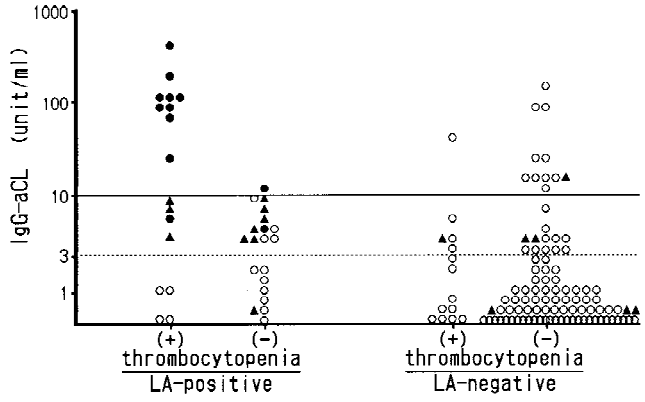

Fig. 1. Relation between thrombocytopenia and the levels of anticardiolipin antibodies (aCL) with or without lupus anticoagulant (LA). Thrombocytopenia was present when the platelet count was below 100 × 10^9/L measured on two separate occasions, ●, with arterial thrombosis; ▲, with venous thrombosis; ○, without thrombosis. The 3.0 U/ml valve of aCL was taken as the normal cut-off point. The levels of aCL below 10 U/ml were regarded as a low level of aCL, and those above 10 U/ml, as a high level of aCL.

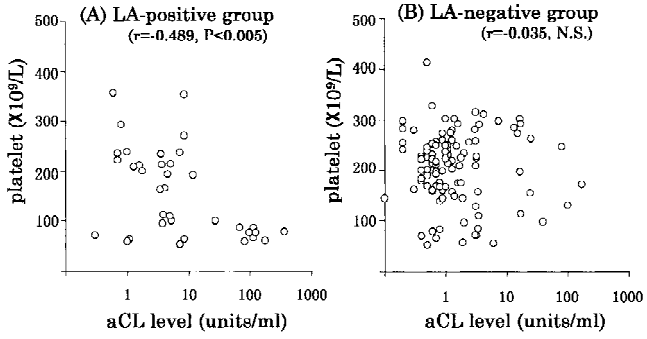

Fig. 2. Correlation between platelet count and anticardiolipin antibodies (aCL) levels in patients with or without lupus anticoagulant (LA). A: LA-positive patient group (n = 36). B: LA negative patient group (n = 107). There was a clear negative correlation between platelet count and aCL levels in the LA-positive patient group (A) (r = −0.489, $P < 0.005$, Pearson's correlation coefficient). No correlation was found between platelet count and aCL levels in the LA-negative patient group (B) (r = −0.035).

Table II. Prevalence of Thrombotic Complications in SLE Patients With or Without Thrombocytopenia

	Thrombocytopenia (+) (%)	Thrombocytopenia (−) (%)	Total
Arterial thrombosis	10 (83.3)[*,**]	2 (16.7)	12
Venous thrombosis	4 (23.5)[***]	13 (76.5)	17
No thrombosis	14 (12.3)	100 (87.7)	114
Total	28 (19.6)	115 (80.4)	143

[*]$P < 0.005$ vs. venous thrombosis, [**]$P < 0.001$ vs. no thrombosis, [***]$P > 0.05$ vs. no-thrombosis, Fisher's exact probability test.

sis (1 deep vein thrombosis, 3 renal vein thrombosis, 13 thrombophlebitis). Moderate thrombocytopenia was present in 10 (83.3%) of the 12 patients with arterial thrombosis, 4 (23.5%) of the 17 patients with venous thrombosis, and 14 (12.3%) of the 114 patients without thrombosis (Table II). There was a significant difference in the incidence of moderate thrombocytopenia between patients with arterial thrombosis and patients with venous thrombosis ($P < 0.005$, Fisher's exact probability test).

DISCUSSION

Thrombocytopenia is one of the common manifestations in patients with SLE, and its incidence has been reported to be as high as 10–40% of them [1,6,12]. It has also been stated that the relationship between elevated IgG-aCL and thrombocytopenia is highly significant, with a statistical correlation between thrombocytopenia and elevated aCL levels for both the IgG and IgM immunoglobulins classes [6], and that IgG-aCL might play a direct role in mediating platelet destruction [6].

Therefore, as a first step we studied the relationship between the level of IgG-aCL and the prevalence of thrombocytopenia in our patients with SLE. The prevalence of thrombocytopenia was higher in the aCL-positive patient group (18/53 cases, 32.0%) than that in the aCL-negative patients group (13/93 cases, 14.0%), as reported by Harris et al. However, we focused on the half of the patients with high levels of aCL who did not have thrombocytopenia.

In a very recent study, we demonstrated that the prevalence of thrombotic complications was much higher in aCL and LA double positive patients than that in aCL or LA single positive and double negative patients [13]. Therefore, we divided our 146 patients into the six groups indicated in Materials and Methods.

The prevalence of thrombocytopenia was by far the highest in group A (9/10 cases, 90.0%) as compared with those in group B (4/15 cases, 26.7%), group C (4/12 cases, 33.3%), group D (1/12 cases, 8.3%), group E (4/16 cases, 25.5%), and group F (9/81 cases, 11.1%). It has been reported that anti-platelet antibodies, more than antiphospholipid antibodies, appear to play a role in the pathogenesis of thrombocytopenia [23,24]. Therefore, we studied the platelet-associated IgG in the 31 patients with thrombocytopenia, and found that three (one patient in group C, two patients in group F) of the 31 patients were not aCL positive, but had platelet-associated IgG. The remaining 28 patients with thrombocytopenia had no detectable anti-platelet antibodies. Three patients with anti-platelet antibodies had severe thrombocytopenia, and this severe thrombocytopenia was considered to be associated with these platelet-specific antibodies.

It is important to note that the prevalence of thrombocytopenia was quite high in group A, and quite low in group D. Furthermore, in the LA-positive patient group, there was a clear negative correlation between platelet count and aCL levels, whereas no correlation was noted between them in the LA-negative patient group. These findings suggest the possibility that the level of aCL might be one of the important factors in the pathogenesis of thrombocytopenia in LA-positive patients; however, only a high level of aCL in LA-negative patients may not be sufficient for the pathogenesis of thrombocytopenia.

It has been reported that the prevalence of thrombosis in SLE patients with moderate thrombocytopenia (platelet count 50–100 $\times 10^9$/L) was similar to that in patients with a normal platelet count and that, conversely, severe thrombocytopenia (platelet count $<50 \times 10^9$/L) was associated with a significantly reduced prevalence of thrombosis [12,24]. Indeed, thrombosis was not found in our three patients with severe thrombocytopenia.

When the relationship between moderate thrombocytopenia and arterial or venous thrombosis was studied in our patients with SLE, we found the prevalence of moderate thrombocytopenia to be significantly higher in patients with arterial thrombosis (83.3%) than that in those with venous thrombosis (23.5%) or without thrombosis (12.3%). It is significant to note that the vast majority of patients who were LA positive and had a high level of aCL not only had thrombocytopenia but also arterial thrombosis. There is a possibility that the cooperation of both a high level of aCL and LA might play a role in mediating platelet activation, aggregation, and destruction. Further studies on activation and destruction of platelets from SLE patients are currently in progress.

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REFERENCES

1. Gladman DD, Urowitz MB, Tozman EC, Glynn MFX: Haemostatic abnormalities in systemic lupus erythematosus. Q J Med, New Series LII 207:424–433, 1983.
2. Harris EN, Gharavi AE, Boey ML, Patel BM, Mackworth-Young CG, Loizou S, Hughes GRV: Anticardiolipin antibodies: Detection by radioimmuno assay and association with thrombosis in systemic lupus erythematosus. Lancet 2:1211–1214, 1983.
3. Hughes GRV, Harris EN, Gharavi AE: The anticardiolipin syndrome. J Rheumatol 13:486–489, 1986.
4. Boey ML, Colaco CB, Gharavi AE, Elkon KB, Loizou S, Hughes GRV: Thrombosis in systemic lupus erythematosus: Striking association with the presence of circulating lupus anticoagulant. Br Med J 287:1021–1023, 1983.
5. Koike T, Sueishi M, Funaki H, Tomioka H, Yoshida S: Anticardiolipin antibodies and biological false positive serological test for syphilis in patients with systemic lupus erythematoses. Clin Exp Immunol 56: 193–199, 1984.
6. Harris EN, Asherson RA, Gharavi AE, Morgan SH, Derue G, Hughes GRV: Thrombocytopenia in SLE and related autoimmune disorders: Association with anticardiolipin antibody. Br J Haematol 59:227–230, 1985.
7. Harris EN, Chan JKH, Asherson RA, Aber VR, Gharavi AE, Hughes GRV: Thrombosis, recurrent fetal loss, and thrombocytopenia. Arch Intern Med 146:2153–2156, 1986.
8. Gharavi AE, Harris EN, Asherson RA, Hughes GRV: Anticardiolipin antibodies: Isotype distribution and phospholipid specificity. Ann Rheum Dis 46:1–6, 1987.
9. Lechner K: Lupus anticoagulants and thrombosis. International Society on Thrombosis and Leuven University Press 525–547, 1987.
10. Galli M, Comfurius P, Maassen C, Hemker HC, De Baets MH, van Breda-Vriesman PJC, Barbui T, Zwaal RFA, Bevers EM: Anticardiolipin antibodies (ACA) directed not to cardiolipin but to a plasma protein cofactor. Lancet 335:1544–1547, 1990.
11. McNeil HP, Chesterman CN, Krilis SA: Immunology and clinical importance of antiphospholipid antibodies. Adv Immunol 49:193–280, 1991.
12. Italian Registry of Antiphospholipid Antibodies (IR-APA): Thrombosis and thrombocytopenia in antiphospholipid syndrome (idiopathic and secondary to SLE): First report from the Italian Registry. Haematologica 78:313–318, 1993.
13. Nojima J, Suehisa E, Akita N, Toku M, Fushimi R, Tada H, Kuratsune H, Machii T, Kitani T, Amino N: Risk of arterial thrombosis in patients with anticardiolipin antibodies and lupus anticoagulant. Br J Haematol 96:447–450, 1997.
14. McNeil HP, Simpson RJ, Chesterman CN, Krilis SA: Antiphospholipid antibodies are directed against a complex antigen that includes a lipid-binding inhibitor of coagulation; b2-glycoprotein I (apolipoprotein H). Proc Natl Acad Sci USA 87:4120–4124, 1990.
15. Koike T, Matsuura E: What is the ''true'' antigen for anticardiolipin antibodies? Lancet 337:671, 1991.
16. Bevers EM, Galli M: b2-glycoprotein I for binding of anticardiolipin antibodies to cardiolipin. Lancet ii:952–953, 1990.
17. Keeling DM, Wilson ALG, Mackie IJ, Machin SJ, Isenberg DA: Some antiphospholipid antibodies bind to b2-glycoprotein I in the absence of phospholipid. Br J Haematol 82:571–574, 1992.
18. Matsuura E, Igarashi Y, Yasuda T, Koike T, Triplett DA: Anticardiolipin antibodies recognize b2-glycoprotein I structure altered by interacting with an oxygen modified solid phase surface. J Exp Med 179: 457–462, 1994.
19. Matsuura E, Igarashi Y, Fujimoto M, Ichikawa K, Suzuki T, Sumida T, Yasuda T, Koike T: Heterogeneity of anticardiolipin antibodies defined by the anticardiolipin cofactor. J Immunol 148:3885–3891, 1992.
20. Schleider MA, Nachman RL, Jaffe EA, Coleman M: A clinical study of the lupus anticoagulant. Blood 48:499–509, 1976.
21. Triplett DA, Barna LK, Unger GA: A hexagonol (II) phase phospholipid neutralization assay for lupus anticoagulant identification. Thromb Haemost 70:787–793, 1993.
22. Tsubakio T, Kurata Y, Yonezawa T, Kitani T: Quantification of platelets associated IgG with competitive solid-phase enzyme immunoassay. Acta Haematol 66:251–256, 1981.
23. Stasi R, Stipa E, Oliva F, Sciarra A, Perrotti A, Olivieri M, Zaccari G, Gandolfo MG, Galli M, Barbui T, Papa G: Prevalence and clinical significance of elevated antiphospholipid antibodies in patients with idiopathic thrombocytopenia purpura. Blood 84:4203–4207, 1994.
24. Galli M: Thrombocytopenia in the antiphospholipid syndrome. Br J Haematol 93:1–5, 1996.